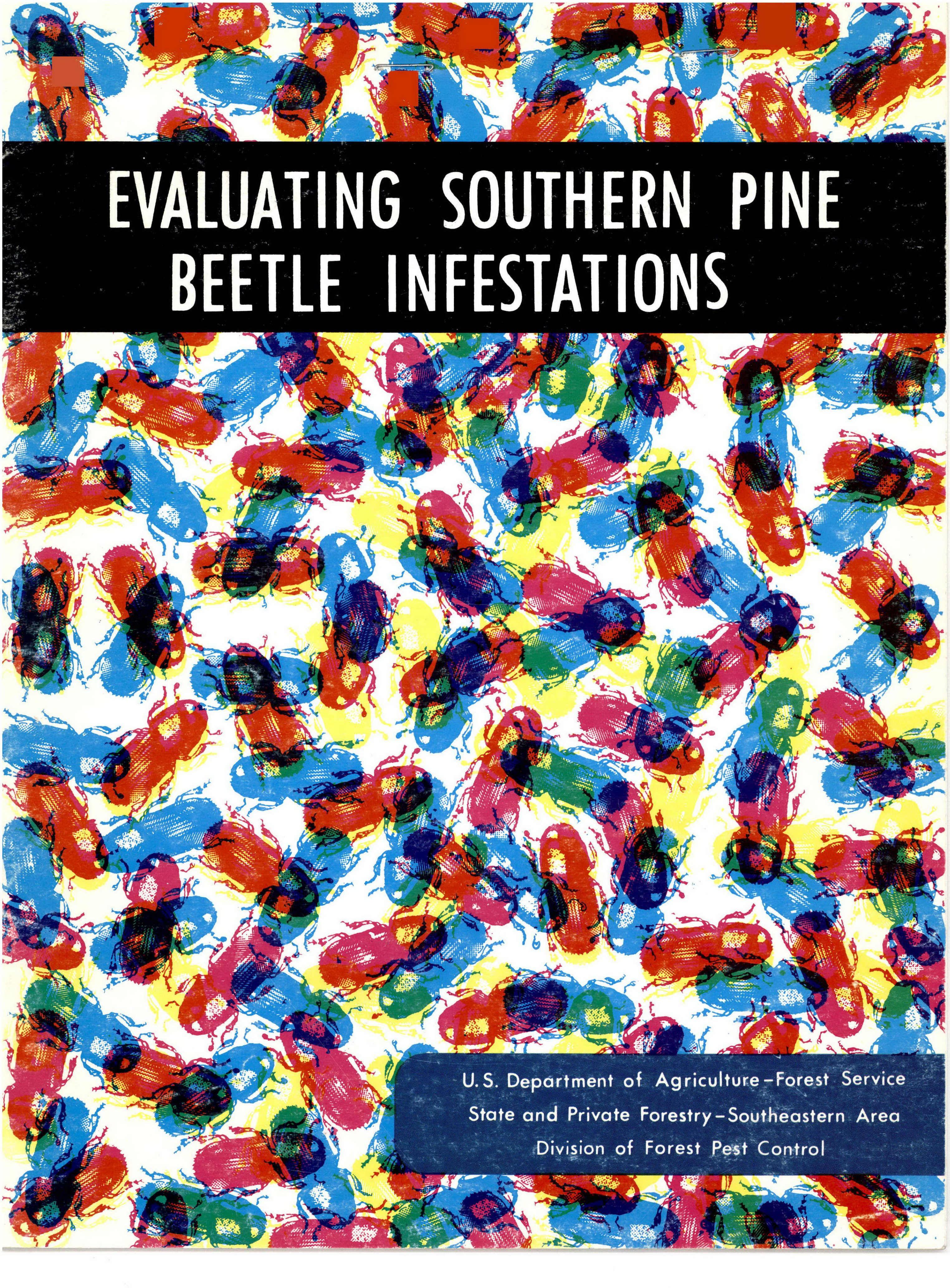
EVALUATING SOUTHERN PINE BEETLE INFESTATIONS



U.S. Department of Agriculture–Forest Service
State and Private Forestry–Southeastern Area
Division of Forest Pest Control

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PREFACE

The intent in this publication is to bring under one cover the more pertinent information concerning the evaluation of southern pine beetle infestations as practiced by the Division of Forest Pest Control, Southeastern Area, U. S. Forest Service. It is recognized that rapid technological advances are being made in equipment and procedures that will necessitate changes in this manual. Revisions of this publication will be made periodically to meet this need. Written notification of any errors or omissions will be appreciated.

INTRODUCTION

The southern pine beetle is one of the most destructive forest insects found in the Southeastern United States. Widespread, spectacular and frequent outbreaks of this pest have been recorded since late in the 1800's - - some encompassing nearly the entire pine type of the southeast. Methods for assessing the losses caused by this insect and the biological factors involved have been greatly improved in recent years. Early estimates were made entirely from ground observations, with aerial observations gaining prominence in the 1950's. In 1962 the use of an aerial operations recorder was initiated which increased the accuracy of the aerial estimates but was restricted to fairly level terrain. The use of aerial photography in assessing southern pine beetle infestations began in 1965 and has proved to be an accurate and rapid method of determining tree losses.

Information on beetle populations and the amount of parasitism or predation has been obtained through the years primarily from bark samples laboriously dissected by hand or by the time consuming rearing of beetle broods in cages. Recently x-rays have come into use to obtain this same information with much greater efficiency, accuracy and economy.

This publication describes the procedures currently in use by the Division of Forest Pest Control, Southeastern Area for assessing southern pine beetle population levels and tree losses caused by this insect pest. The procedures involve primarily the use of color infrared aerial photography of infestation areas and x-rays of bark samples.

AERIAL PHOTOGRAPHIC SURVEYS

Aerial color photography is a valuable tool for measuring infestation levels of the southern pine beetle **Dendroctonus frontalis** Zimm., for making control decisions, for determining the magnitude of a project to effectively suppress epidemic populations and to follow population trends in outbreak areas. This technique has a number of advantages over other survey methods which have been used in the past including accuracy -- photographs can be studied in detail, thus eliminating errors of omission inherent in visual observation from low flying aircraft; versatility -- the photographic technique may be used in mountainous as well as level terrain; safety -- photographic surveys are made from higher altitudes; and efficiency -- the aerial photographs provide an accurate map showing the location of infested areas; consequently, the amount of time spent in ground checking is significantly reduced.

The aerial photographic survey technique is a double sampling procedure consisting of aerial sampling units and ground sample units. The following data are obtained from this survey procedure:

1. Number of spots (groups of discolored pines) per 1000 acres susceptible host type.
2. Number of actively infested trees per 1000 acres susceptible host type.

Susceptible host type is defined as any forested area where at least 25 percent of the stems are pine.

Photographic surveys, designed to measure the level of southern pine beetle infestation, consist of a number of separate and distinct operations as follows:

1. Planning the photographic mission.
2. Flying the photography.
3. Processing the film.
4. Photo interpretation.
5. Ground checking
6. Analysis of data.

These procedures can be conducted by any individual with a basic knowledge of photogrammetry, photo interpretation, and color film processing, and who has normal color vision.

The following are a series of guidelines and standards which may be used to design and conduct aerial photographic surveys of southern pine beetle infestations. These guidelines are based on experience in using this survey techinque throughout the 13 states which comprise the Southeastern Area of the U. S. Forest Service and should be applicable with only minor changes anywhere the southern pine beetle is a pest.

PLANNING THE PHOTOGRAPHIC MISSION

Defining the area to be surveyed.--The general boundaries of the infestation should be defined prior to measuring population levels within the area. This may be done quite readily by an aerial sketchmap reconnaissance survey over areas where beetle activity has been reported. This preliminary survey should be flown at an altitude of approximately 1,000 feet above the terrain at an airspeed of 90-100 mph.

When the general infestation boundaries have been established, the area should be blocked off into discrete boundaries if possible (Fig. 1). The area within these boundaries in terms of square miles and acres should then be determined.

Number of aerial photo sample plots.--A minimum of 30 sample plots is required to obtain reliable estimates of southern pine beetle population levels. Using this procedure, sample plots are distributed systematically over the established area of infestion on a series of predetermined flight lines, generally in an east-west direction. The sample plot consists of a stereo pair of aerial photographs (60 percent overlap) which constitutes the basic sampling unit. The number of sample plots to be taken is dependent upon the total area of infestation, the percent sample desired and the size of sample plot. It is desirable to obtain a 2-to-10 percent sample of the area of infestation. Consequently, for an infestation comprising 500,000 acres, one should include from 10,000 to 50,000 acres in the sample. Size of the individual sample plots is dependent upon photo scale. Three photo scales have been used for photographic surveys of southern pine beetle infestations in the Southeastern United States (Table 1.).

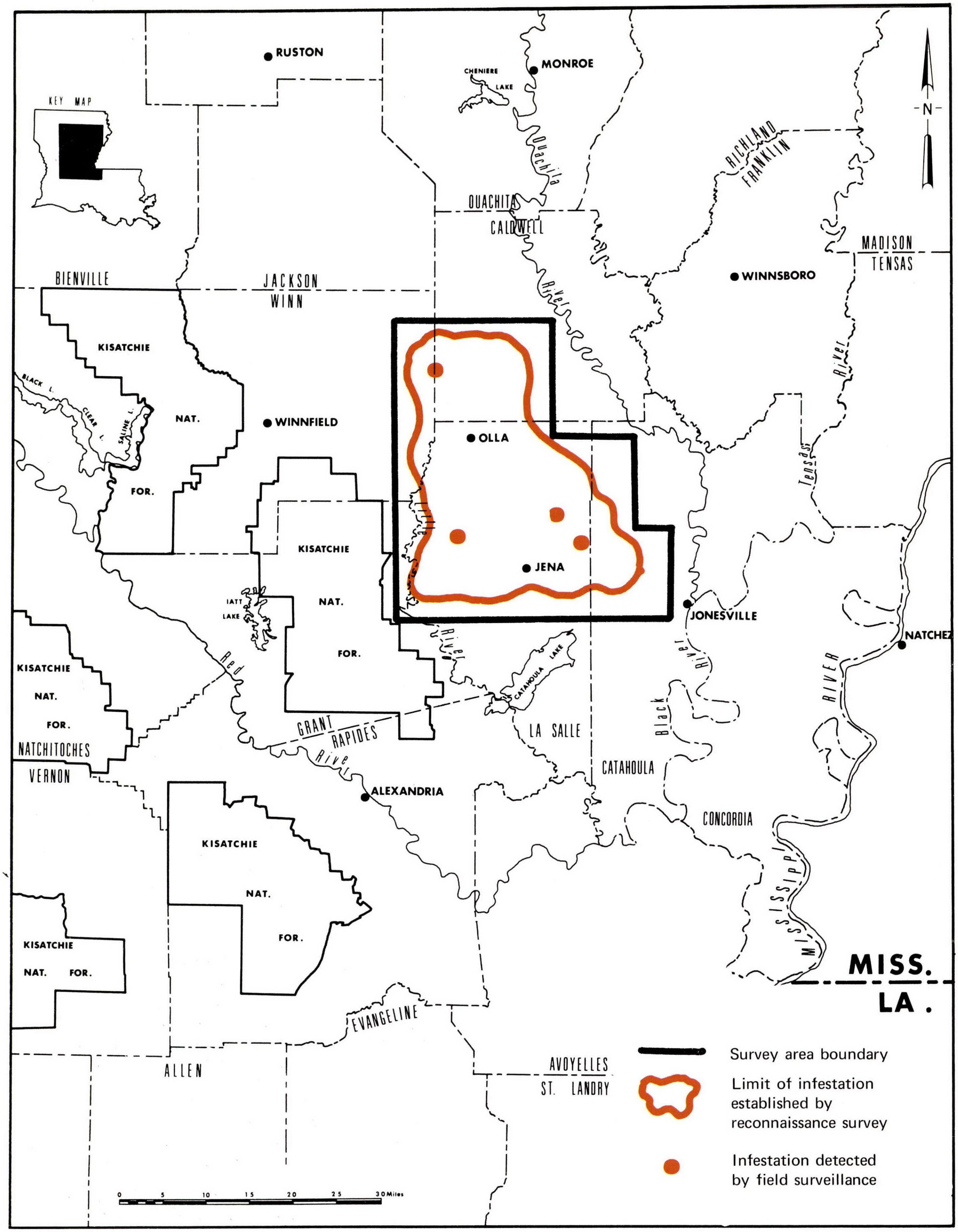

Figure 1. Blocking off areas of southern pine beetle infestation into discrete units.

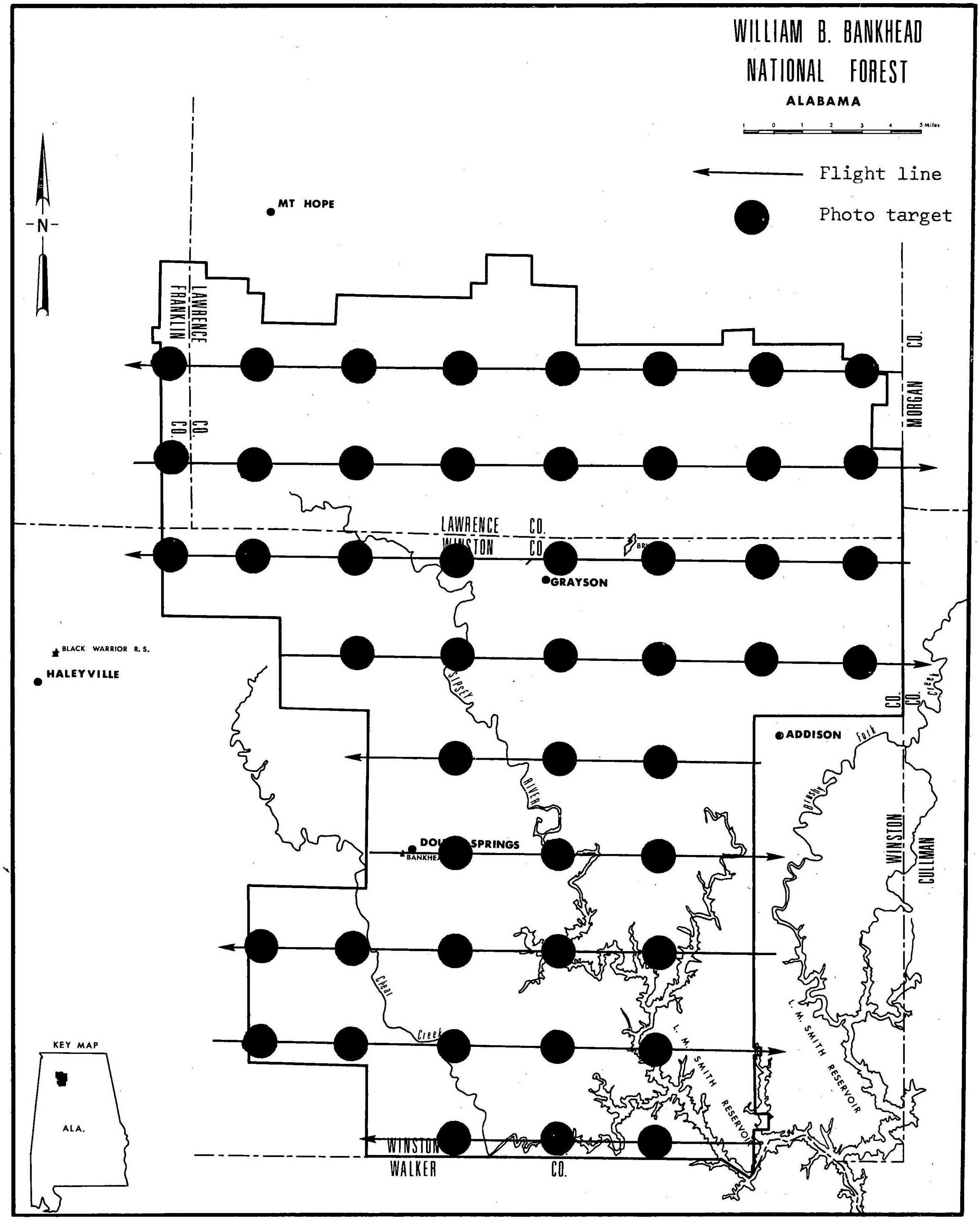

Figure 2. Typical layout of flight lines and photo targets over a southern pine beetle infestation.

Table 1. Photo scales, plot sizes and dimensions for southern pine beetle photographic surveys.

Photo Scale	Plot Size (Acres)	Plot Dimensions (Inches)
1:3960 (16 in. = 1 mi.)	50	4 x 5
1:5940 (12 in. = 1 mi.)	200	4.5 x 7.44
1:7920 (8 in. = 1 mi.)	200	4 x 5

Experience has shown that a photo scale of 1:5940 is optimum for the Southeastern United States in that it provides a large sample plot and the photo scale is still sufficiently large to resolve individual tree crowns with considerable detail.

Another consideration in determining the number of sample plots is the number of plots which can be obtained from one roll of film. Aerial color film is available in 9½ in. x 125 or 200 ft. rolls. One hundred and sixty-six exposures can be obtained from one 124 ft. roll of aerial film which is sufficient to photograph 83 sample plots (two exposures/plot). From an operational standpoint, it is advantageous to expose an entire roll of aerial film once it is loaded into the camera magazine. For example, if a 5% sample is taken of a 500,000 acre area, a total of 25,000 acres is to be sampled. If 200 acre sample plots are used, a total of 125 plots is required for a 5% sample. Two rolls of aerial film would provide coverage of 166 sample plots or 6.6% coverage. It is more desirable to take the larger sample rather than expose a partial roll of film.

Flight line and photo plot interval. – Spacing of flight lines and photo plots along each flight line may be obtained from the following relationship:

$$I = \sqrt{\frac{A}{N}}$$

Where: A = total area to be surveyed in sq. miles or sq. meters.

N = number of photo plots.

I = flight line interval and photo plot interval in miles or meters.

For example, if 100 photo plots are to be taken over a 500,000 acre outbreak area (781 square miles) then:

$$I = \sqrt{\frac{781}{100}} = \sqrt{7.81} = 2.8$$

Therefore, the distance between flight lines and the interval between photo plots is 2.8 miles.

Preparation of survey maps. – When the number of photo plots and the interval between photo plots and flight lines has been established, the flight lines and photo plots should be laid out on a map of the area to be surveyed (Fig. 2). Almost any planimetric or topographic map can be used for this purpose. A rule of thumb is to use the best map coverage that is available. In the Southeastern United States, U. S. G. S. quadrangle maps (scale 1 in. = 1 mi.) or aerial photo index mosaics are used.

Aerial films. – A great variety of aerial films are commercially available for various photogrammetric applications. Heller et al. (1959) established that color aerial film was superior to panchromatic black and white film for detecting southern pine beetle infestations. Ciesla et al. (1967) reported that Ektachrome infrared Aero film (Type 8443) was superior to a standard color film for photographic surveys of southern pine beetle infestations. Ektachrome infrared Aero film is a false color film with three emulsion layers sensitive to green, red and near infrared portions of the electromagnetic spectrum (.9 microns) (Fritz, 1967). All layers are highly sensitive to blue light, therefore, this film is used in combination with a Wratten No. 12 (minus blue) filter. Ektachrome Infrared Aero film, in combination with a minus blue filter, penetrates atmospheric haze and is capable of differentiating coniferous foliage from deciduous

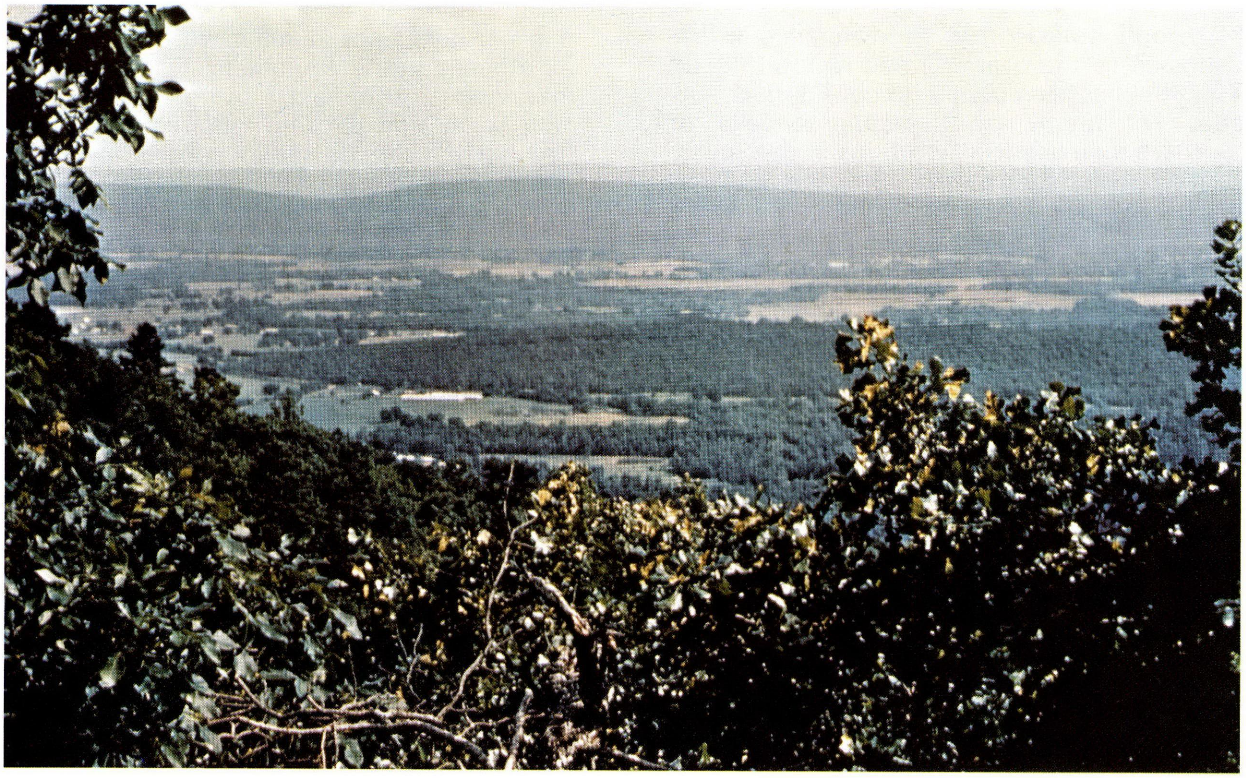

Figure 3. Paired photographs taken with a normal color film (upper) and Ektachrome Infrared Aero film (lower) showing haze penetrating qualities of Ektachrome Infrared Aero film and its ability to separate pines from hardwoods. Brilliant red areas are hardwood forests, darker areas are pines.

hardwood foliage due to difference in reflectance in the near infrared region (Fig. 3). This film has been used with considerable success in aerial photographic surveys of southern pine beetle infestations in the Southeastern United States since 1966. An ASA rating of 160 has produced optimum exposures for this application.

An estar base film, Kodak Aerochrome Infrared film (Type 2443) is now available which replaces Ektachrome Infrared Aero film (Type 8443).

FLYING THE PHOTOGRAPHY

Equipment requirements. — Equipment required to fly photographic missions includes a suitable aircraft, aerial camera, camera magazines, intervalometer, and viewfinder. Almost any aircraft equipped with a camera hatch for taking vertical aerial photographs will suffice. In mountainous terrain, light, single-engine aircraft may be more subject to turbulence which can introduce errors in the scale of the photography. Experience has shown that the 500 series Aero Commanders are ideally suited for aerial photography because of overhead wing visibility, multi-engine safety, and the fact that they provide a very stable platform from which to take aerial photographs.

An aerial camera capable of taking 9 x 9 in. format aerial photographs is recommended. The large format usually provides imagery of enough land area to contain sufficient landmarks to tie the photograph back to its location on the ground in the event that it is selected for ground checking. A Fairchild K-17 aerial camera is suitable for this purpose. The resolution capability of the camera lens is adequate to produce the imagery required. In addition, the K-17 is relatively simple to operate. This is a decided advantage when using personnel not familiar with sophisticated photogrammetric equipment. It is desirable to have two to three extra film magazines available so that all the film required to complete the survey can be taken aboard the survey plane ready to use.

The focal length of the camera lens cone is a critical factor in determining the flying height of the survey aircraft above the terrain to obtain the desired photo scale. The longer the focal length of the lens cone, the higher the flying height required to obtain the desired scale. Lens cones with longer focal lengths produce aerial photographs with a minimum of edge distortion. However, in high mountainous terrain, it is often desirable to use a shorter lens cone to avoid having to fly at altitudes where supplemental oxygen is required. Most aerial cameras are equipped with either a 6, 8¼, or 12-inch lens cone. Flying heights above mean terrain elevation for each of the photo scales are shown in Table 2. Aerial cameras equipped with 12-inch lens cones have been used to the greatest extent in the Southeastern United States.

An intervalometer is required to make exposures at the correct interval to provide 60% overlap between stereo pairs. The Fairchild B3B intervalometer is designed for use with a K-17 aerial camera. A vertical viewfinder, placed directly behind the camera, is used to determine the proper interval between exposures to provide the desired 60% overlap. The interval setting is determined by measuring the time required for an object to move across the graduated ground glass plate in the view-finder. This can be easily determined with a stopwatch. The reading, in seconds, obtained from the viewfinder, is set on the intervalometer.

Table 2. Flying height in feet above mean terrain elevation to obtain desired photo scale.

Focal Length of Lens Cone	Desired Photo Scale 1:3960	1:5940	1:7920
	Height in feet		
6 in.	1,980	2,970	3,960
8¼ in.	2,275	4,087	5,449
12 in.	3,960	5,940	7,920

Manpower requirements. — A two-man crew, in addition to the pilot, consisting of a tracker and a cameraman is necessary to conduct the photographic mission. The tracker, seated next to the pilot, keeps the pilot on the flight line and watches for the upcoming photo targets. The cameraman is seated directly behind the camera. His duties include determining the interval setting at the beginning of each flight line, keeping the camera level when photos are being taken, activating the intervalometer when the tracker informs him that the survey aircraft is over the photo target and taking light meter readings at periodic intervals during the photo mission to determine optimum exposure.

Climatic factors. — Weather is a critical factor in the success of a photographic mission. Clear, cloudless days are best for aerial photography. Atmospheric haze is readily penetrated by Ektachrome Infrared Aero film even when haze restricts visibility to less than one mile. Ektachrome Infrared Aero film will not penetrate larger particles such as those in smoke or clouds, however. Low altitude cumulus clouds are a deterrent to photographic missions in that they are generally beneath the altitude of the survey plane and cause cloud shadows on the ground which can result in portions of the aerial photograph being grossly underexposed (Fig. 4). Cirrus clouds which generally occur at 10-12,000 feet are less of a deterrent to photographic missions.

Time of day is an important consideration in taking aerial photographs. For best results, photographic missions should be flown near mid-day (1000 hrs. — 1430 hrs.) when the sun angle approaches 90°. As the sun nears the horizon, long shadows tend to obscure details on the photographs. This is particularly critical over mountainous areas where entire slopes might be shaded.

FILM PROCESSING

Detailed instructions for processing color aerial film are provided with each roll of film. The procedure, although time consuming, is not difficult and aerial photographs of high quality can be obtained if instructions are closely adhered to.

Directions for processing Kodak Aerochrome Infrared film 2443 in rewind equipment are taken directly from Eastman Kodak Data Release M-69. (See Tables 3 and 4).

Figure 4.

Oblique aerial photograph showing effect of low altitude cumulus clouds on photo quality.

Processing Chemicals

The Kodak packaged chemicals shown in the following chart are required for rewind processing.

TABLE 3

Kodak Chemicals for Rewind Processing of KODAK AEROCHROME Infrared Film 2443 (ESTAR Base)		
Name	Gallon Size	No. of Pkgs.
KODAK Prehardener and Replenisher, Process E-4	3½	2
KODAK Neutralizer MX-875*	3½	2
KODAK Neutralizer Additive MX-870*	3½	2
KODAK First Developer, Process E-4	3½	2
KODAK Stop Bath and Replenisher, Process E-4	3½	4
KODAK Color Developer, Process E-4**	3½	2
KODAK Bleach and Replenisher, Process E-4	3½	2
KODAK Fixer and Replenisher, Processes C-22, E-3, E4	3½	2
KODAK Stabilizer and Replenisher, Processes E-3, E-4	3½	2

*Mix two 3½-gallon size units of KODAK Neutralizer MX-875 according to package directions. Add two 3½-gallon size units of KODAK Neutralizer Additive MX-870 and dilute with water to make 7 gallons of neutralizer working solution.

**Color developer pH must be adjusted by addition of 2.5 milliliters of 7N (280 gm/1) sodium hydroxide solution per liter of developer. Seven gallons of developer solution will require 66 milliliters of 7N sodium hydroxide.

To make 1 liter of sodium hydroxide, 7N, slowly add 280 gm of KODAK Sodium Hydroxide, Granular, to 800 ml of water. Mix until dissolved. After cooling to room temperature, add water to bring total volume to 1 liter and mix for 5 minutes. **Caution:** Sodium hydroxide (caustic soda) is a poison. See caution notices given on labels of containers. Always add sodium hydroxide to water; **never** add water to sodium hydroxide. Do not weigh sodium hydroxide in an aluminum dish.

TABLE 4

Processing Sequence (9½-inch x 125-foot rolls):

Processing Step	Time (Minutes)	Temperature
Prehardener	5	85 ± 1 F
Neutralizer	1	85 ± 2 F
First Developer	9	85 ± ½ F
First Stop	2	85 ± 2 F
Wash	4	85 ± 2 F
Color Developer	15	85 ± 2 F
Second Stop	3	85 ± 2 F
Wash	3	85 ± 2 F
Bleach	10	85 ± 2 F
Fixer	10	85 ± 2 F
Wash	8	85 ± 2 F
Stabilizer	1	85 (approximately)
Dry	—	—

Processing the Film (Aim Temperature 85 F)

- Fresh solutions should be used for each roll of film and should be discarded after use.
- The film must be agitated by continuously winding it from one reel to the other while it is in each of the solutions and each time it is rinsed or washed.
- Before the reel assembly is advanced from one solution to the other, the following operation must be performed:

  Lift the reel assembly out of the solution, turn the reel until the film is *taut,* and then tip the entire assembly over far enough (more than 90 degrees) to permit any solution held in the pockets within the film convolutions or mechanism to run out. Hold the film and reel in this position for approximately *10 seconds* in order to drain out most of the solution. *The drain time should be included in the time specified for each processing step.*

- The timing or counting of each step begins *after* the film is placed in the solution and is wound from one reel to the other so that the film will be uniformly wet with the solution before timing begins.

1. Preharden the film in the prehardener at 85 ± 1 F for 5 minutes as follows:
   a. *In total darkness,* wind the film (emulsion side out) from the camera spool onto one of the processor reels. Attach the tail of the film securely to the processor reel, as required for rewinding.
   b. With the reels horizontal and the empty reel at the bottom, lower the processor reel assembly into the prehardener tank until the empty reel is totally submerged. *Do not allow the upper reel and the film to become wet.* It may be necessary to devise a way to hold the processor reel assembly in this position.
   c. Slowly wind the film completely onto the submerged reel, maintaining a constant speed. Lift the processor reel assembly out of the tank and place it in its normal operating position, with both reels vertical and totally submerged in the prehardener.
   d. Place the motor in position, start it, and begin timing the prehardener step. Near the end of the 5 minute period, let the motor roll the film onto one of the reels. Remove the assembly from the prehardener tank and drain the film and reel assembly.

2. Neutralize the film in KODAK Neutralizer MX-875 at 85 ± 2 F for 1 minute.

3. Develop the film in the first developer at 85 ± ½ F for 6 minutes. *The development step is critical and timing should be accurate within a few seconds.*

   Near the end of the development time, let the motor roll the film onto one of the reels; remove the assembly and drain the film. Include the drain time in the total development time.

A motor-speed control is recommended so that the film will be wound onto one reel near the end of the development time.

4. Stop the development by treating the film in the first stop bath at 85 ± 2 F for 2 minutes. Room lights may be turned on after this step.

6. Develop the film in the pH-adjusted color developer at 85 ± 2 F for 15 minutes.

5. Wash the film in running water at 85 ± 2 F for 4 minutes.

7. Stop the development by treating the film in the second stop bath at 85 ± 2 F for 3 minutes.

8. Wash the film in running water at 85 ± 2 F for 3 minutes.

9. Bleach the film in the bleach solution at 85 ± 2 F for 10 minutes.

   CAUTION: The bleach corrodes most metals and, therefore, should not be left in metal equipment any longer than is absolutely necessary. Bleach can, however, be stored in receptacles made of red brass, polyethylene, porcelain, rubber, or glass; it may also be stored in enamelware receptacles that are free from surface cracks or chips.

10. Fix the film in the fixer at 85 ± 2 F for 10 minutes.

11. Wash the film in running water at 85 ± 2 F for 8 minutes.

12. Stabilize the film in the stabilizing bath at approximately 85 F for 1 minute.

13. Dry the film in a dust-free area. To prevent sticking when the film is wound into rolls, it is important that both sides of the processed film be dried thoroughly. The film should be dried in equilibrium with 45 to 55 percent relative humidity. This will minimize size changes.

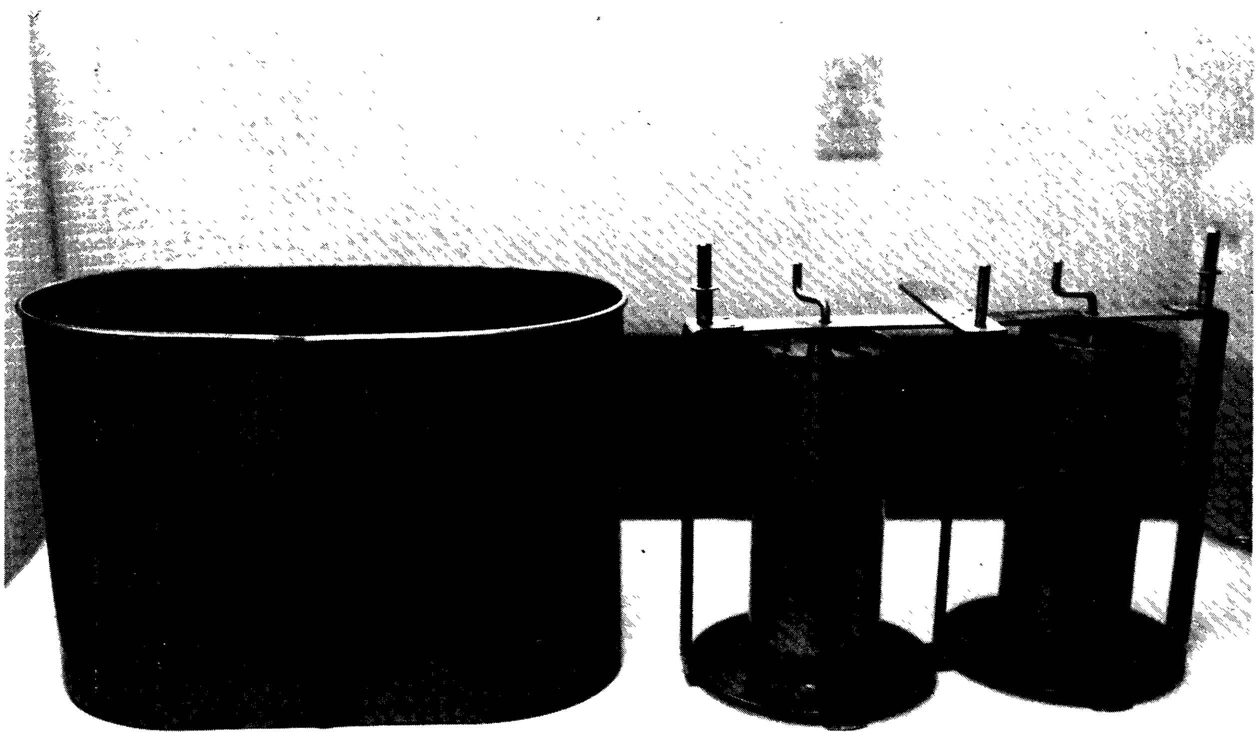

Figure 5. Five gallon processing tank and rewind process reel for processing aerial film.

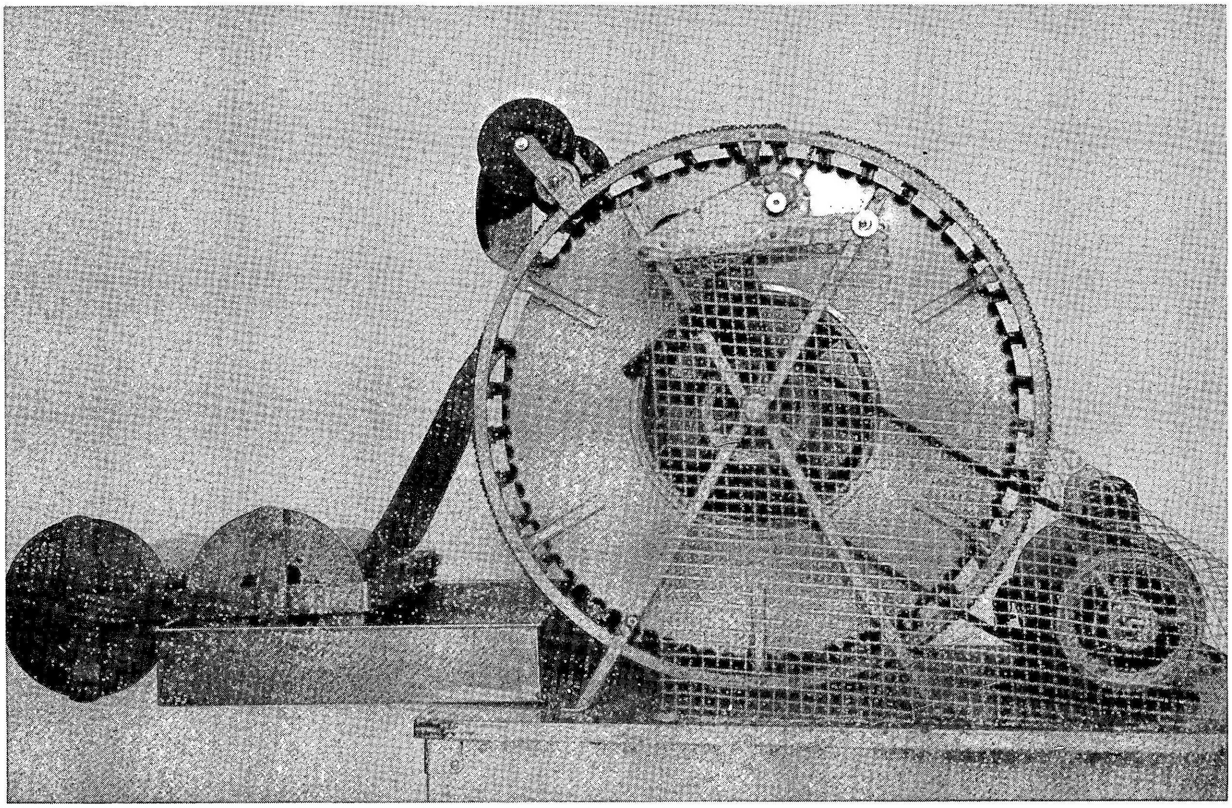

Figure 6. Aerial film dryer.

PHOTO INTERPRETATION

Equipment Required. – Photo interpretation may begin as soon as the color transparencies have dried. Equipment required to interpret color transparencies includes a mirror stereoscope, preferably a scanning type such as an Old Delft, a light table, acreage grids and china markers (preferably white).

Locating photo pairs. – The first step in photo interpretation is to plot the actual location of each stereo pair on the survey map. There is a good likelihood that air currents will cause the photo plane to occasionally drift off course, resulting in the photographs being of some area other than the intended photo target. If the survey area has abundant landmarks and the survey map contains considerable detail, this can be done quite readily. It is necessary to know the exact position of each photo pair in the event that an infestation appearing on that pair is selected for ground checking. Each stereo pair of photographs should be numbered consecutively for identification as they are located on the survey map.

Determining area of susceptible host type. – When the stereo pairs are located on the survey map, the sample plots are placed in the stereo overlap portion of each pair of photographs using a white china marker. The acreage of susceptible host type is then determined for each sample plot using a standard modified acreage grid (256 dots per square inch) or a grid specifically designed for southern pine beetle surveys (Fig. 7). Susceptible host type is defined as any stand where 25% or more of the stems are pine. Areas of coniferous forests can readily be distinguished from deciduous hardwood stands because conifers tend to appear as dark purple or brown images whereas deciduous hardwoods appear as brilliant red images (Fig. 8). The acreage of susceptible host type for each plot is entered on a summary data sheet (Fig. 9).

Locating barkbeetle infestations. – The final step in the photo interpretation process is locating areas of barkbeetle infestation on the sample plots. This is done by scanning each plot stereoscopically and searching for discolored crowns (Fig. 10). Photo interpreters, working with Ektachrome Infrared Aero film must have some knowledge of the characteristics of this film to interpret the various colors which appear on the transparencies.

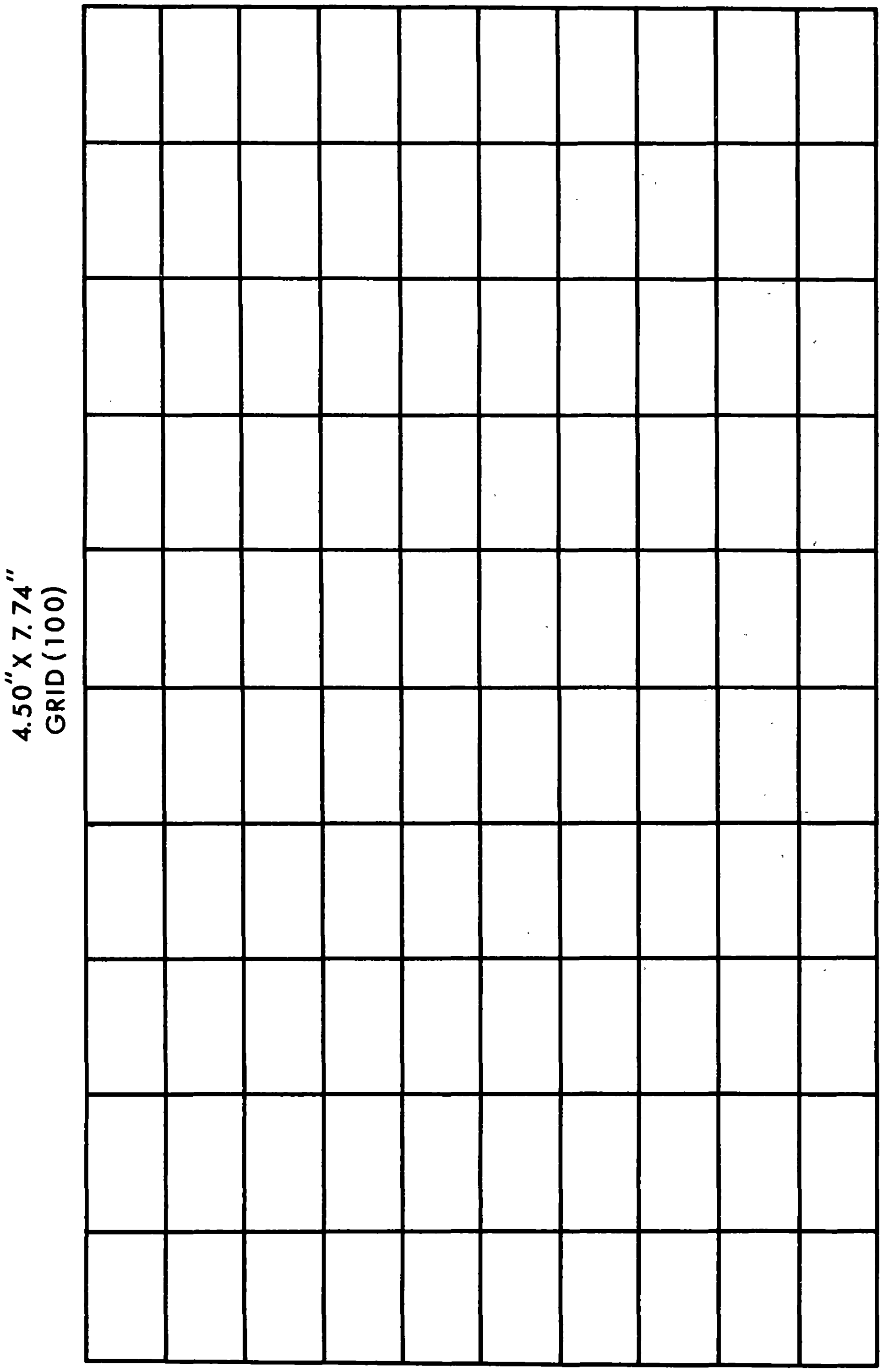

Figure 7. Grid for estimating acreage of susceptible host type on an aerial photo sample plot. Each grid = 2 acres.

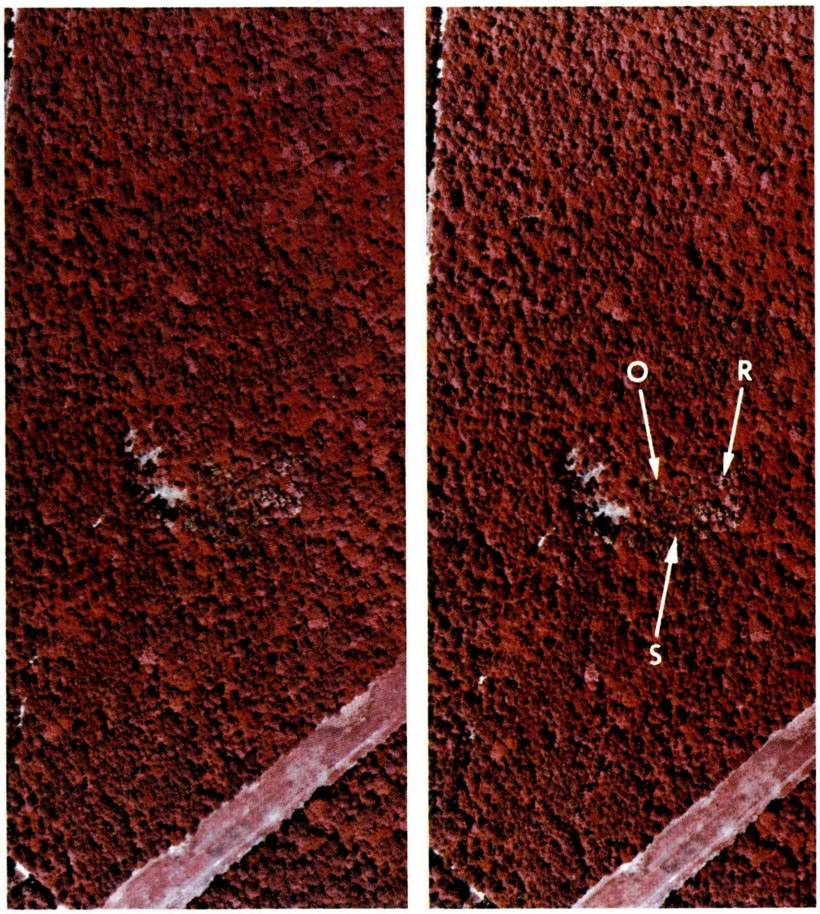

Figure 8.

Aerial stereogram of a southern pine beetle infestation taken with Ektachrome Infrared Aero film at a scale of 1:5960. Light pink crowns (R) are recently dead pines, yellowish crowns (O) are older dead pines and grey to green crowns (S) are pine snags which have lost most of their foliage.

AERIAL PHOTOGRAPHIC SURVEY DATA SHEET

Survey Location ____________. Film ______________________________

Dates Flown ____________. Photo Crew __________________________

Plot #	Acres Host Type	Total No. Spots	No. Red Tops & Faders/Spot					Photo Interpreter
			1	2	3	4	5	

Figure 9. Sample data sheet for summarizing photo interpretation data.

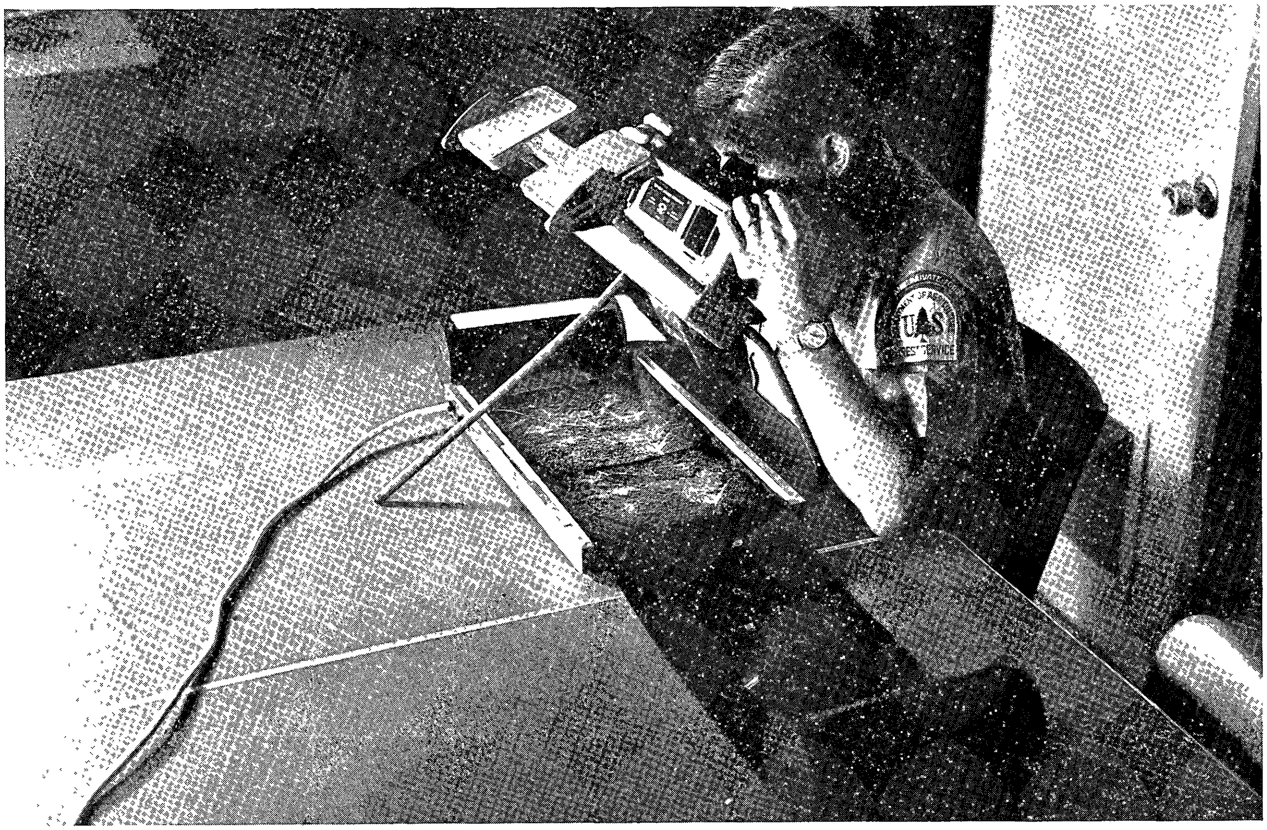

Figure 10. Photo interpreter scanning sample plot with a mirror stereoscope on a portable light table.

Objects highly reflective in the infrared region, such as healthy vegetation, appear in varying shades of red. Green colored objects which do not reflect in the infrared region, such as artificial or diseased foliage, appear as a blue color. The guide following Figure 11 may be used to interpret colors which appear on Ektachrome Infrared Aero film.

The southern yellow pines, when infested by bark beetles, typically undergo a change in foliage color from green to yellow to red as the tree dies. This color change appears on Ektachrome Infrared Aero film from dark red or magenta to beige to yellow. Photo interpreters should look for groups of beige or yellow colored crowns on Ektachrome Infrared Aero film (Fig. 8). These are indicative of active southern pine beetle infestations (Table 5). Evidence to date indicates that previsual symptoms of bark beetle infestation (green infested pines) can not be detected on Ektachrome Infrared Aero film.

Each infestation located on the sample plots should be numbered for identification and a rough numerical estimate of discolored crowns in the infestation should be made. The following numbering system is currently being used to identify spots on aerial photo surveys made in the Southeastern United States:

1 - 1
2 - 1
2 - 2 etc.

The first number refers to sample plot number and the second number refers to the individual infestation. The above data are recorded on the "Aerial Photographic Survey Data Sheet" (Fig. 9).

Table 5. Interpretation guide to foliage colors on Ektachrome Infrared Aero film as related to condition of the tree and status of southern pine beetle brood development in the Southeastern United States.

Crown Color on Ektachrome IR Transparency	Condition of tree	Probable Status of **D. frontalis** Brood 1/
Brilliant red	Healthy deciduous hardwood	
Purple or dark brown	Healthy or recently infested pine	Attacking adults, early instar larvae
Beige or tan	Fading pine	Larvae, pupae, late instar larvae
Yellow	Red-topped pine	Pupae, callow adults. Some emergence occurring
Yellow green	Red-topped pine prior to needle fall	Brood emerged
Blue green-grey	Snag	Brood emerged

1/ Subject to seasonal variation.

A B

Figure 11. *Comparison of color on normal and Ektachrome Infrared Aero film. A. Normal color photograph of a chameleon, note how it blends with the surrounding foliage. B. Ektachrome Infrared photograph of a chameleon. The chameleon's pigment does not reflect in the infrared region, hence, the blue image on the film. The surrounding foliage does reflect in the infrared region, however, producing a red image. C. Artificial foliage against a back drop of normal healthy foliage on normal color film. D. Same photo taken with Ektachrome Infrared Aero film. Note difference in color of artificial and real foliage.*

C D

COLOR COMPARISON

NATURE	EKTACHROME IR AERO FILM
Green or blue, highly IR reflective	Bright Red
Green or blue, low degree of IR reflectance	Dark Red or Magenta
Red, IR reflective	Yellow
Red, non-IR reflective	Green
Green or blue, non-IR reflective	Blue

SUMMARY
GROUND-CHECKING DATA

Location of survey: ____________________ Summarized by: ____________________

Date made: ____________________ Date: ____________________

Spot number	Number of infested trees (y)

Figure 12. Sample data sheet for summarizing ground check data.

GROUND CHECKING

A number of infestations must be field checked to obtain data on the causal agent responsible for the tree mortality and the actual number of trees containing active southern pine beetle infestations. Only symptoms of an abnormality are detectable on aerial photographs; in this case,(discolored crowns) supplemental ground checks must be made to identify the causal agent. In addition, Ektachrome Infrared Aero film, or any other remote sensor developed to date, is not capable of detecting green infested trees; therefore, counts of the number of actively infested trees must be made on the ground in several spots to obtain an estimate of the number of infested trees per 1000 acres host type.

Determining the number of spots to be ground checked. -- Field checking bark beetle infestations, particularly in remote areas, is a tedious, time consuming task; however, the variation in infestation size inherent in southern pine beetle outbreaks, makes it necessary to ground check a considerable number of infestations. A rule of thumb has been devised which is a compromise between statistical validity and what is practical; if less than 30 infestations are detected on the sample plots, ground check all of the infestations. If more than 30 infestations are detected, randomly select 30-50 infestations for ground checking without regard to infestation size or accessibility.

Use of aerial photos for field checking. – The time spent in locating an infestation can be reduced significantly by using the aerial photos as a map of the location of that infestation. Rigid clear plastic holders are available for carrying aerial photographs into the field. Ground survey crews can orient themselves by holding the transparencies toward the sky for study.

Data to be collected. – The primary causal agent responsible for tree mortality in each area should be identified if possible. If the causal agent is the southern pine beetle, each tree should be examined to determine if it contains active brood by carefully shaving the outer layers of bark. The total number of trees with active southern pine beetle broods should be recorded for each infestation ground checked.

In very large infestations (100 trees or more) it is impractical to examine every tree for the presence of an active infestation. An alternative method is to run a transect through the infestation and sample 50-100 trees. A rough count of the number of green infested, fading, and red-topped pines should then be made. Apply the proportion of actively infested trees in the transect sample to the total number of trees in the spot to obtain an estimate of actively infested trees. Data from each infestation field checked may be summarized on a form similar to that shown in Fig. 12.

The association of factors such as lightning, wildfire, poor drainage, logging activity, overmature timber and other environmental or man caused factors should also be recorded. These data might provide the survey entomologist and the forest land manager with an insight into the role of environmental influences which tend to favor the development of epidemic populations of this insect in a given outbreak area.

ANALYSIS OF DATA

Data obtained from photo interpretation and ground checks may be analyzed in accordance with procedures described by Ketcham (1964) to provide estimates of:

1. **Number of spots per 1000 acres host type.**
2. **Number of infested trees per 1000 acres host type.**

Number of spots per 1000 acres host type-- Summarize data from the "Aerial Photographic Survey Data Sheet" (Fig. 9) as follows:

Number of spots (x)
Area of susceptible host type (a)
Number of sample plots (n)

The number of spots per acre (r) and its variance (S_r^2) and the standard error (S_r) are computed as follows:

1. $r = \dfrac{\Sigma x}{\Sigma a}$

2. $S_r^2 = \dfrac{n}{(n-1)(\Sigma a)^2}\ [\Sigma x^2 + r^2\,\Sigma a^2 - 2r\Sigma ax]$

3. $S_r = \sqrt{S_r^2}$

The number of spots per 1000 acres (R) and its standard error (S_R) are computed as follows:

4. $R = 1000r$

5. $S_R = 1000S_r$

Number of infested trees per 1000 acres host type.--Summarize data from the ground check summary data sheet as follows:

Number of actively infested trees (y)
Number of infestations ground checked (n_1)

The number of infested trees per acre (i), its variance (S_i^2) and standard error (S_i) are computed from the following equations:

6. $$\bar{y} = \frac{\Sigma y}{n_1}$$

7. $$S_{\bar{y}}^2 = \frac{\Sigma y^2 - \bar{y}\Sigma y}{n_1(n_1 - 1)}$$

8. $$i = r\bar{y}$$

9. $$S_i^2 = r^2 S_{\bar{y}}^2 + \bar{y}^2 S_r^2$$

10. $$S_i = \sqrt{S_i^2}$$

The number of infested trees per 1000 acres (I) and its standard error (S_I) are computed as follows:

11. $$I = 1000i$$

12. $$S_I \quad 1000S_i$$

Precision.--Confidence intervals for the number of spots per 1000 acres (R) and number of infested trees per 1000 acres (I) are computed as follows:

13. $$R \mp S_R t$$

14. $$I \mp S_I t$$

Where: t = value for a specified level of precision. If estimates are desired to be correct nine times out of ten (90% confidence level), the 0.1 values of t for n-1 degrees of freedom should be used.

Decision criteria.--The number of actively infested trees per 1000 acres susceptible host type is used as an index to decide for or against control. In the Southeastern United States, if infestation levels are in excess of 2.00 trees per 1000 acres host type, it is generally recommended that control measures be initiated.

RADIOGRAPHIC SAMPLING OF BROOD DENSITY

Reliable estimates of immature southern pine beetle brood density provide the best means of predicting population trends and future damage by the southern pine beetle. Many bark beetles in the genus **Dendroctonus** spend their entire developmental period at the cambium/phloem interface where they can be studied by simply removing the bark. In contrast, the brood of the southern pine beetle **Dendroctonus frontalis** Zimm. mines away from the cambium/phloem interface and completes its development in the outer bark. To get a reliable estimate of the brood density within an infestation area enough bark samples must be examined to get a representative sample. Estimates of brood density may be obtained by dissecting bark samples or by counting the brood on radiographs of bark samples.

Dissection of bark samples is an extremely time consuming and tedious task accomplished by paring off thin layers of bark and counting the immature insects as they are encountered. Studies by DeMars (1963) and Fatzinger and Dixon (1965) indicate that 2.6 minutes was required to dissect a square inch of bark sample; or thirty-seven minutes to dissect a 1/10 square foot sample. Based on the 5" x 6" bark sample most commonly used by the Division of Forest Pest Control, radiography requires .165 hours per sample and dissection 1.30 hours per sample. In other words, eight samples can be analyzed by radiography in the time it takes to dissect one bark sample. The time per sample for radiography (.165 hours) includes exposing, processing and interpreting the radiographs. In actual application, this time will vary depending on the uniformity of the samples and the efficiency of the radiographic system. Aside from the initial cost of the x-ray machine and processing equipment, a sample can be radiographed for less than one-fifth the cost of dissection. For the data to be useful to entomologists and land managers, it is imperative that the samples be rapidly and accurately analyzed. This is especially true when working with a multi-generation insect subject to wide variation in population density.

Previous workers using both medical and portable low KV x-ray units have determined correlation coefficients for the number of insects found on radiographs compared to (1)

the number found by dissection (DeMars 1963) and (2), the estimated true total number of insects in the bark sample (Fatzinger and Dixon 1965). The highest correlations were obtained in comparing the total number of insects present where Fatzinger and Dixon reported a correlation coefficient of .96. When interpreters were required to separate larvae from pupae and adults, the correlation coefficients dropped to an average of .85. These figures are a guide as to what can be expected in actual practice. However, the actual figure will depend on the quality of the radiographs and the experience of the interpreter.

Initial inspection of the radiographs may be concerned with only the total number of insects and the predominant stage of development. In this respect the radiographs offer a wealth of information.

Bark dissection destroys the sample. All the data must be recorded upon initial examination. The radiograph of a bark sample provides a permanent record not only of the number of developing insects but also the gallery pattern, predator population and the distribution of the insects within the sample. If adequate records are kept to identify the sample, data can be collected from the radiograph at a later date for a variety of studies.

An x-ray machine is an expensive piece of equipment. The least expensive x-ray machines suitable for biological studies range in price from $1,500 to $6,000. Additional equipment must be purchased and darkroom space provided for processing the radiograph. Medical x-ray machines at local hospitals have been used by investigators in the past (Johnson and Molatore, 1961 and Fatzinger and Dixon, 1965) but are not as well suited for biological studies as industrial low kilo-voltage x-ray machines. While medical x-ray machines have been used in pilot studies, it is unlikely that a hospital would agree to their use on a long term basis.

Operators of x-ray machines must receive special training to insure that established safety standards are met. With some x-ray machines protective lead shield enclosures must be built if the machine is to be operated in the laboratory. Because of the expense of equipment and specialized training, the purchase of an x-ray unit can be justified only if the expected use justifies the purchase. In determining expected use, other forest insects such as seed and cone insects, wood borers, etc., should be considered along with its use in determining southern pine beetle brood density. Where the purchase cannot be justified, the sending of samples to be radiographed at a laboratory with existing radiographic capabilities should be explored.

COLLECTION OF SAMPLES

What constitutes a representative sample is one of the hardest questions to answer in a biological sampling program. The design of a sampling procedure depends on the distribution, variation and density of the insect population. For radiographic brood sampling of southern pine beetle, most of these questions remain to be worked out.

Knight (1960) developed a sequential brood density sampling plan to predict trends in Black Hills beetle populations. In sequential sampling, the decision to collect another sample depends on the total count of insects found in previous samples. This system is not directly applicable in radiographic surveys where all the samples must be collected and returned to the laboratory before any counting can be done.

A sampling plan was developed by the Division of Forest Pest Control (Ketcham and Williamson, Unpublished) for evaluating the reproduction ratio of the southern pine beetle. This plan called for the collection of samples from three trees in each of five spots. Three samples each were collected from the top, middle and bottom portion of the infested stem. This plan is more suitable for pilot studies than functional biological evaluations.

Considering the variability in southern pine beetle populations a fairly large sample is required to provide a statistically valid estimate of southern pine beetle brood density. In the plan currently used for biological evaluation of southern pine beetle infestations distribution of samples is based on the premise that a more representative sample can be obtained by reducing the number of samples per tree and increasing the number of spots and trees from which samples are collected. In each evaluation, samples are collected from six trees per spot in thirteen spots, for a total of 78 samples collected.

Figure 13. Removing bark sample from southern pine beetle infested tree.

SAMPLE SIZE

The size of the individual bark sample removed from infested trees is an important factor in determining the accuracy of brood density estimates. The current collection method consists of one 5 x 6 inch bark sample from each sample tree. Samples of uniform, regular dimensions are easiest to store and radiograph. Rectangular samples are most easily obtained by using a hand ax and a rubber mallet to cut the bark. (Fig. 13) A sheath knife is helpful in separating the bark sample from the tree. The back of the hatchet handle can be marked with paint as a guide in cutting the proper sample size.

The one-tenth square foot circular bark sample was the standard size in the Division of Forest Pest Control sampling plan prior to acquiring an x-ray unit. In some cases samples as small as 1/20 square foot were used. Small samples are generally easier to remove from the tree and are easier to dissect. In radiographic studies larger samples are easier to handle and identify.

Studies were made to determine the best sample size for western bark beetle species by Carlson and Cole (1965), and Knight (1959). Both studies indicated that larger samples provided a more consistent estimate of brood density. Knight's sequential sampling plan calls for samples 6" x 6" (.25 square feet).

Preliminary Southern pine beetle radiographic studies have been carried out using superimposed sample sizes ranging from .382 square feet to .050 square feet. At the high brood densities (481 insects per square foot) all sample sizes had similar means. The total range of sample brood densities recorded for individual samples increased with decreasing sample size. Smaller sample units are most likely to be misleading at low brood densities or when only a small number of samples are collected.

LOCATION OF SAMPLE

In the current sampling plan samples are collected in a zone from four to six feet above the base of the tree. One sample from this zone is removed from each sample tree. Sampling the base of the tree below four feet is not recommended because of the inconsistency of beetle attacks. Sampling the upper portions of the bole of infested trees is not feasible in functional biological evaluations. Felling sample trees is time consuming and is likely to bias the sample in favor of smaller and more accessible trees. Brood density is only intended to provide an index of reproductive activity. In most situations a significant portion of the population will deposit their eggs in the lower portion of the bole where samples can be collected.

The sampling universe in southern pine beetle brood density surveys is all currently infested trees within the survey area. For this reason, only successfully attacked trees in currently active spots should be selected for sampling. The presence of beetle broods should be verified before removing a sample. This requires cutting into the bark of trees with a hand ax to expose the insects.

FIELD DATA COLLECTION

It is imperative that forms used in data collection be complete, providing all the data necessary for detailed data analysis at a later date. The forms should present the data in a logical sequence in such a manner that they can easily be read by the operator of the calculator or computer encoder. Figure 14 presents the form currently used by the Division of Forest Pest Control. The same form is used in the field, laboratory and in preliminary data analysis. At the completion of the survey, the data sheets are filed with radiographs for future reference. The data sheet, along with a set of instructions (Fig. 15) and codes, has been successfully used in large scale biological evaluations.

The sheet provides sufficient data for roughly plotting the spot on maps. Additional data are collected on stand conditions and timber volume which can be used in estimating economic loss and also for impact studies.

STORAGE AND TRANSPORTATION OF SAMPLES

Bark samples must be properly stored if they are to provide useful radiographs. The money and time expended in sample collection are wasted if the samples are not properly stored. Bark samples should be kept moist and cool. The brood will die and may be undetectable on radiographs if the samples are allowed to dry out. Callow adults may emerge if bark samples are too warm.

Samples should be placed in individual plastic bags to prevent desiccation and loss of brood. Air sick sacks make ideal storage bags for bark samples. Spot and sample number may be written directly on the bag with a ball point pen. These bags are provided with wire tie closures that will prevent samples from drying out. All the bags with samples from the same spot should be kept together either with a rubber band or in a paper bag.

During the summer samples should be transported in a cooler chest with regular or dry ice. The temperatures that build up in a closed automobile are sufficient to kill the brood.

Special shipping containers and shipping instructions can be obtained for shipping samples by public transportation. Either air freight or bus freight should be used where possible. Dry ice or chipped ice in a closed container should be used in the shipping container to prevent overheating during shipment. Shipments should be scheduled to arrive at their destination during the regular work day. The field office should be notified by telephone of incoming shipments.

Samples can be safely stored in the refrigerator for several days before being radiographed. However, samples will dry out if kept for long periods in a refrigerator. Samples may be frozen if they cannot be radiographed within a few days of receipt. Samples should be radiographed immediately after being removed from the freezer because frozen samples allowed to defrost will quickly deteriorate.

Figure 14.

SOUTHERN PINE BEETLE BARK SAMPLES – DATA SHEET FOR FIELD AND LABORATORY

Individual Spot Data

Spot#	Location: Co.	Location: Photo No.	Date Sample Taken: Mo.	Date Sample Taken: Day	Date Sample Taken: Yr.	Number of trees per spot by color: G	F	R	B	Av. DBH	Av. Ht.	Basal Area	Average brood density per sq. ft of Bark	Site Characteristics Slope Definition: Ridge top	N	E	S	W	Low Land

Sample Tree Data

Sam. No.	Tree Species	DBH Sam. Tree	Ht. Sam. Tree	Color Sam. Tree	% Bark removed by woodpecker	X-ray Exposure Number	Major Stage of Life Cycle	Brood Density Per Sample	Size of sample in Sq. Ft.	REMARKS

Shaded area to be completed in lab.

Key to Data Sheet for Collecting Southern Pine Beetle Bark Sample

I. Individual Spot Data (See attached example)

1. Spot # - This should be the number of the spot within a particular county. The spots should be numbered consecutively as collected. If the spot number is a single digit number preceed it with a 0, such as 01, 02, etc. All blocks on form should have a digit in them.

2. Co. - This should also be a number. See attached sheet for particular county code number.

3. Photo No. - The location of each spot should be recorded by a six digit number from photo index sheet of county.

4. Date Sample Taken - See example.

5. Number of trees per spot by color - G - green, F - fader, R - red, B - black. Black would be trees within the spot which have no needles. In each block put the approximate number of trees in each of these four classes. Please place the small letter a by the active trees. They will in most cases be in either the green or fader class.

6. Average DBH - Record here the estimated average D.B.H. for the entire spot.

7. Average Height - Record the estimated average height for the entire spot.

8. Basal Area - Basal area should be taken for each spot. A handy way of doing this would be with a prism.

9. Average brood density per square foot of surface bark - This will be completed at the Division of Forest Pest Control, U.S. Forest Service Office in Asheville, N.C.

10. Site Characteristics, Slope Definition - A check should be placed in only one of the six boxes. N,E,S,W, describes the slope.

II. Sample Tree Data

1. Sample No. - This is the number of the sample tree within the spot.

2. Tree Species - See attached sheet for species code number.

3. DBH Sample Tree - Close estimate of diameter of the sample tree.

4. Height of Sample Tree - Close estimate of height of sample tree.

5. Color of Sample Tree - The colors are coded on attached sheet, place the number representing the color class in the box.

6. % Bark removed by woodpeckers - Enter here the percent of bark completely removed by the woodpeckers. Bark must be removed to the bare wood.

7. The shaded area will be completed in the lab in Asheville, N.C.

Figure 15.

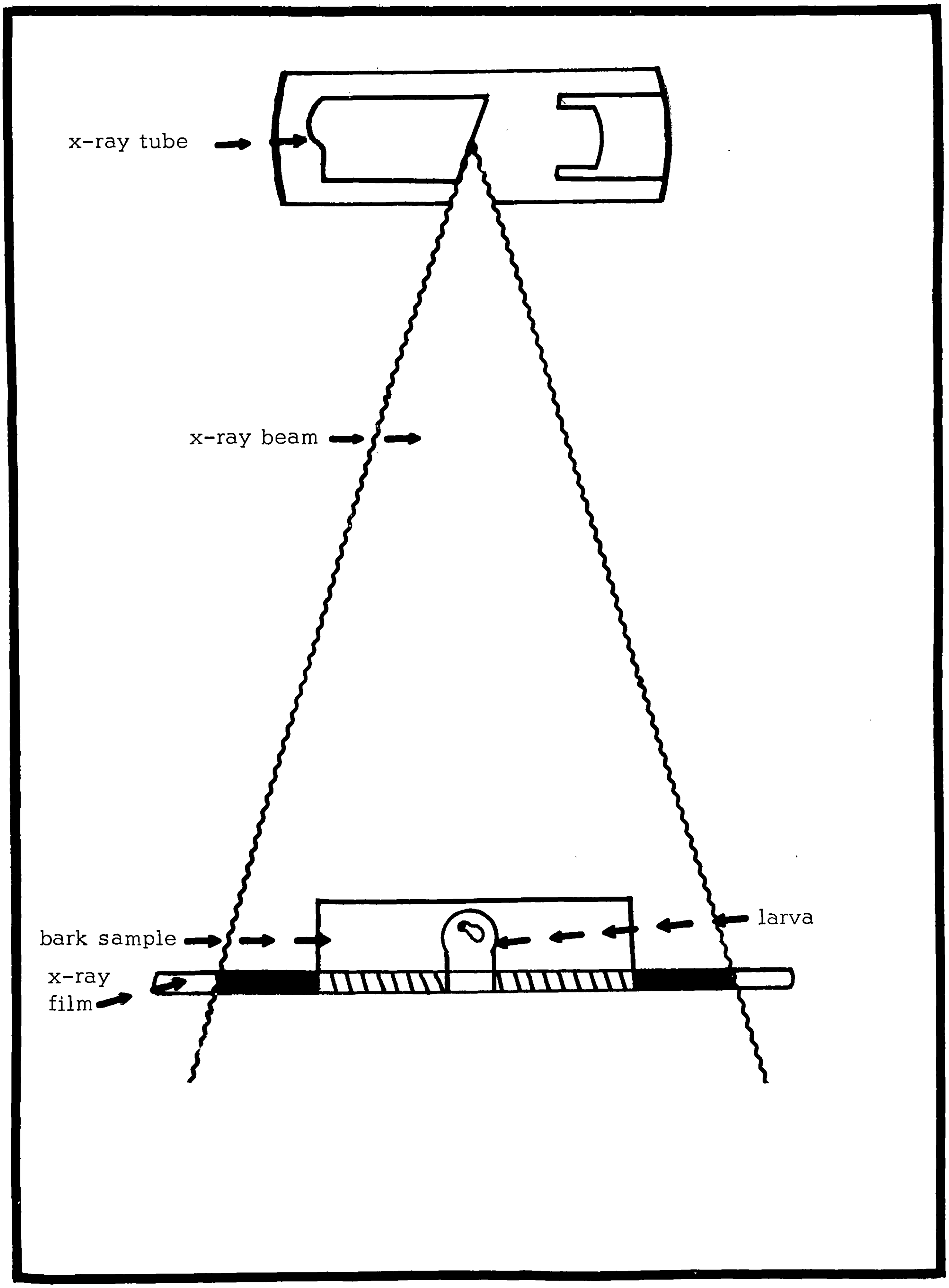

Figure 16. Schematic diagram showing radiographic exposure of a bark sample.

RADIOGRAPHING BARK SAMPLES

Principle of Radiography. — X-Rays, like visible light, are a form of radiant energy. The extremely short wavelength of x-rays permits them to penetrate materials which reflect visible light. They are produced when electrons hit or collide with a target. X-rays form a small proportion of the energy released by this collision; the remainder is given off as heat. Radiographs record on film those x-rays that pass through the sample. Only a portion of the x-rays penetrate the sample; the remainder are absorbed or deflected in their passage through the sample. The penetration of the sample, in our case a bark sample, by x-rays depends on the density, thickness and atomic number of the materials involved (Fig. 16). The bark is composed of materials of low atomic number in contrast to the beetles which contain a greater proportion of elements with higher atomic numbers (nitrogen and inorganic elements). Bark beetle galleries can be distinguished on the radiograph because these portions of the bark are thinner and absorb less of the x-rays striking the sample.

In radiographing bark samples, several samples are usually radiographed on a 10 x 12 inch sheet of film in one exposure. All the samples radiographed in any one exposure should be approximately the same thickness. If the samples vary greatly in thickness, the thicker samples will be underexposed.

SAFETY PROCEDURES

X-ray machines can be operated safely only if adequate precautions are taken and safety instructions are followed. The same properties that permit x-rays to penetrate sample material make them a source of potential danger to the operator. The Forest Service Manual (Section 4064) sets forth procedures that must be complied with prior to the purchase of an x-ray unit. Additional guidelines and procedures to insure the safety of personnel operating x-ray machines are promulgated by the Radiological Safety Committee, U. S. D. A.

The energy released as x-rays passes through living tissues and causes damage to the living cells. Part of the damage caused by exposure to relatively small doses of x-ray are repairable. Repeated exposures cause effects to accumulate and become unrepairable. The symptoms of radiation damage may not show up until the damage caused by many doses has built up over a period of years.

There are several ways to protect the operator of x-ray machines from radiation. X-rays are produced only when exposures are being made. Lead shielding is the primary protection for the operator of an x-ray machine. Lead shielding is built into the cabinets of enclosed industrial x-ray units, while a lead enclosure must be built when portable x-ray units are used in the laboratory. Setting up a restricted safety zone around the machine further reduces the danger of accidental exposure (Fig. 17).

Figure 17. X-ray machine and safety zone, Asheville Office, Division of Forest Pest Control.

Safety rules recommended by the manufacturer and unit safety officer should be posted on the machine as a constant reminder to the operator (Fig. 12). Adequate universal warning signs should be provided to warn persons entering the laboratory that the x-ray machine is in use (Fig. 16). The operator is responsible for the safety of other personnel in the area. He should never leave the x-ray machine unattended while it is in operation.

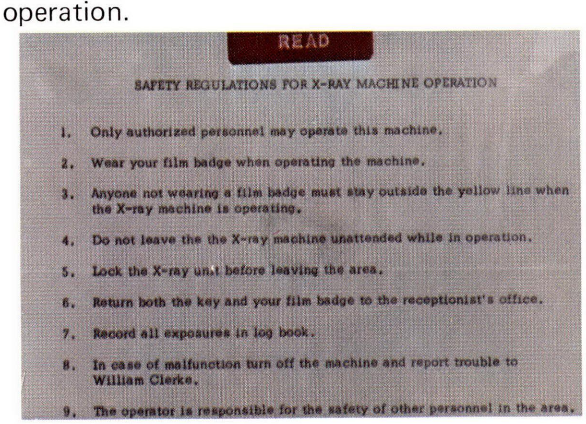

Figure 18. Safety instruction posted on the X-ray machine.

Figure 19 Film badge dosimeters and film packet.

The U. S. D. A. requires that the operator of radiation emitting devices wear film badge dosimeters (Fig. 19). Since x-rays expose photographic film, these badges are a sensitive indicator of the dosage the operator has received. Each operator must have his own film badge and should wear it whenever working in the vicinity of an operating x-ray machine. The film badge should be worn on a part of the body most likely to receive radiation, i.e., shirt pocket or belt. To be most effective, the badge should always be worn with the film window facing away from the wearer. Film badges should be stored with the key to the x-ray unit in an area where they cannot be exposed to radiation. A control badge is provided by the supplier to monitor film packets in transit and storage. It should never be worn by personnel using the x-ray machine. The badges contain two film packets. The first of these, which is changed every two weeks, measures dosage received during short periods of time (Fig. 19). The second film packet, which is changed every six months, measures smaller, long-term dosage that would be undetectable on the two week film packet. The supplier keeps complete records on dosage received by film badge users. Notification will be sent when film packets show detectable exposure.

The federal government sets maximum radiation exposure limits. The limit set for whole body exposure of occupationally exposed persons is five roentgens per year, or 100 mr per week. This is the limit for persons involved in operating the x-ray machine. The Department of Agriculture has set a limit of 25 mr per week, one-fourth the approved dosage limit for personnel operating x-ray equipment.

The x-ray exposure log is the key to radiographic records. Every exposure made with the x-ray machine must be recorded in the log book. Each radiograph should be numbered for identification. This can be best accomplished by placing small lead numbers on the film with the specimen prior to making an exposure. The exposure number, subject, operators initials, date, film, focus distance, KV and milliamperage, film type and machine mode should be filled in at the time of exposure. After the radiographs have been examined, remarks concerning the quality of the radiograph may be added. The exposure log serves several important functions. In the area of safety it serves as a record of the dosage to which the operator has been subjected. The record serves as a basis for evaluating x-ray tube life. Records of exposure data combined with remarks on the results give a less experienced operator an indication of what settings would produce the best exposure of an unfamiliar subject. The log also serves as an index to the radiograph file.

SELECTION OF X–RAY FILM

Selection of x-ray film should be based on the subject, x-ray machine specification and processing system. X-ray films are similar to photographic films but designed to be exposed by x-rays rather than light. In most cases the films are coated with emulsion on both sides. The films are divided into two categories: (1) medical film, generally with high speed, and low contrast and (2) industrial film, with slower speeds and higher contrast. For bark sample radiography, a film in the medium (Type AA) speed group is recommended. Kriedel (unpublished) reports that for seed radiography the increase in definition from medium speed film to a low speed film such as Kodak M is insignificant. For most applications, 10″ x 12″ is an ideal film size (Fig. 20). It is large enough to hold six one-tenth square foot or four two-tenth rectangular samples. The larger the sheet of film the further it must be placed from the x-ray source to be fully covered by the x-ray beam. A sheet of 14″ x 17″ film would require at least a one-third longer exposure time than would be required to expose a full sheet of 10″ x 12″ film. The smaller film size is easier to handle in processing, interpreting, and storing. To be properly exposed, all of the samples on any sheet of film should be approximately the same thickness. When a 14″ x 17″ film is covered with bark samples, they are often sufficiently different in thickness to under-or over-expose some samples.

X-ray film is sensitive to light as well as radiation. The individual sheets of film must be enclosed in light-proof holders before they

Figure 20. Bark samples on Ready-Pac film with numbering block ready to be x-rayed.

are exposed. The thinner the holder, the closer the subject will be to the film. Thick cardboard holders used in medical radiography are not especially suitable for low KV radiography of biological subjects. Film may be purchased in ready-to-use light proof envelopes, with a tear strip for fast unloading (Fig. 20). The paper envelope is more suitable than cardboard holders for low KV radiography. The higher initial cost of ready-to-use film is offset by the time saved in loading holders.

PROCESSING RADIOGRAPHS

Careful processing of the exposed radiographs is essential if they are to yield a maximum of useful information. If processing is standardized, differences in radiographs may be attributed to exposure technique and adjusted to produce the desired results. Improperly processed radiographs will have less detail; important features will be hidden by processing marks and streaks. Radiographs not properly fixed or washed will deteriorate and in time be entirely useless. The steps involved in processing radiographs are not complicated. Good results will be obtained if the directions are followed and cleanliness is maintained to avoid contamination of the processing chemicals. Film should be processed as soon as possible after exposure. The quality of the latent image will be degraded if the film is kept longer than 24 hours after exposure. Heat, chemical fumes and moisture will fog the film and reduce the quality of the image.

Processing systems for radiographic film vary in sophistication and price. The choice of a processing system should be based on the budget and needs of the agency. Automatic processors are primarily intended for high capacity application such as medical laboratories. They are expensive ($7,500) and built for high temperature processing of medical x-ray film. These units are not suitable for most biological applications.

With the exception of automatic processors, all x-ray processing systems involve loading individual sheets of film in holders and manually transferring the holders to tanks holding processing solutions. In most cases the tanks are dimensioned to hold standard 14 x 17 inch film holders. The typical commercially available processing system of this type will hold about five film holders. Removable insert tanks for developer and fixer are placed within a central tank. Water circulating through the central tank is used to wash and rinse radiographs and control the temperature of the chemical solutions. A processing set up of this type should cost about $150.

A RADIOGRAPH PROCESSING SYSTEM FOR OPERATIONAL BIOLOGICAL EVALUATIONS

Neither of the previously described processing systems is ideal for operation on large-scale bark sampling operations. Operational sampling of southern pine beetle infested bark samples requires the rapid evaluation of radiographs. To solve this problem a processing set up was designed by the Asheville Office, Division of Forest Pest Control (Fig. 21). The system is an adaptation

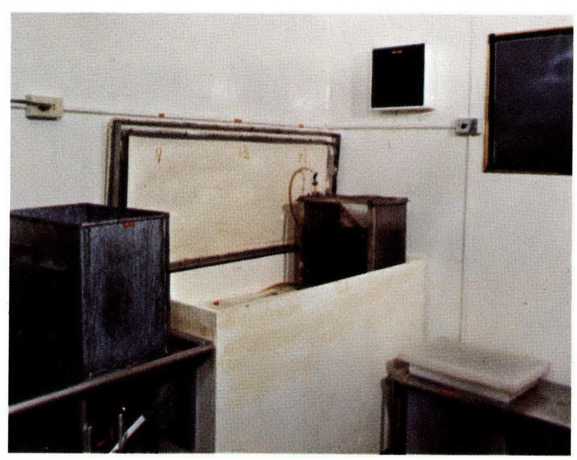

Figure 21. Radiograph processing system developed by the Division of Forest Pest Control.

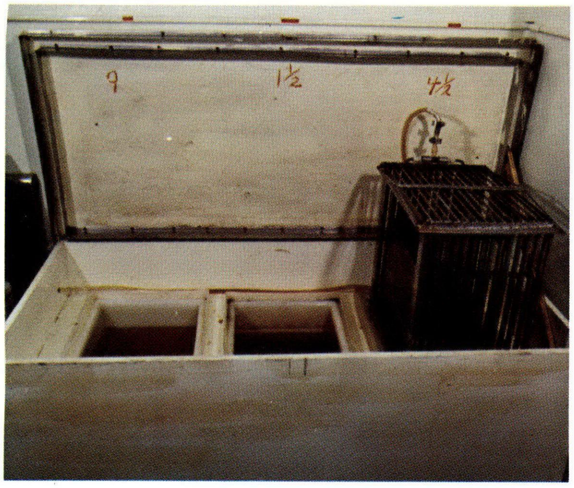

Figure 22. Light tight processing box with gas agitated film rack.

of production sheet film processing designed primarily for processing 10 x 12 inch radiographic film in a minimum of time. The sheets of film are placed in individual custom designed film holders. As the holders are loaded, they are placed in individual slots in a rack designed to hold fourteen film holders.

The entire rack, rather than individual film holders are transferred between tanks (Fig. 22). The time required for each of these processing steps varies with the temperature of the solutions. Suggested processing times are included on the data sheet accompanying the film.

Fifteen gallon polyethylene tanks with floating lids hold the processing chemicals (developer, stays, and fix). The three tanks are supported in a plywood light-tight box. This permits the operator to work in room light, except when transferring the processing rack. An epoxy finish protects the developing box from the processing chemicals.

A nitrogen gas burst grid in the bottom of the developing rack provides agitation for the x-ray film. A two stage regulator on the supply cylinder provides nitrogen to the system at 7 to 10 psi. The burst timer provides a one-second burst of nitrogen every 10 seconds during the film processing. The fixing agent is removed from the film by a two tank wash system (Fig. 23). The rack is transferred from the fix to the rinse tank. After a two minute rinse the film is placed in a double strength solution of Perma-wash for two minutes followed by a two minute water rinse. The addition of Photo Flo to the final rinse will prevent streaking of the radiographs and reduce drying time.

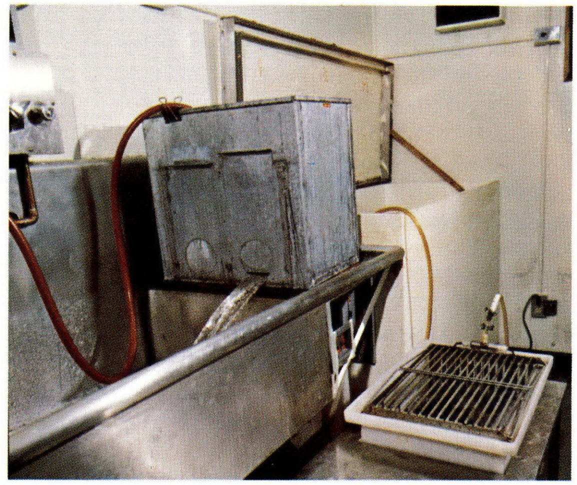

Figure 23. Washing radiographs, quick damp rinse tank and Perm-Wash tank.

RADIOGRAPH PROCESSING CHEMICALS

It is best to become thoroughly familiar with one brand of chemicals rather than using products of several manufacturers. X-ray processing chemicals are available both as powders and liquids. Liquid concentrates are easier to mix and replenish and are preferable in most cases.

The ability of developer to produce a given amount of film darkening is reduced as the number of films developed in the solution increases. This occurs as a result of dilution and oxidation of the developer solution. Oxidation can be kept to a minimum by using floating lids and keeping the developer tank covered when not in use. Developer activity can be maintained by adding a measured amount of replenisher solution for each film processed. Where x-rays are not being developed every day, it is usually sufficient to change developer once every three months.

There is no direct way of measuring developer activity. Activity of the developer can be checked by periodically developing radiographs of a standard sample radiographed under specified conditions. A wood or metal step block makes a good target. One test should be made when the solution is first mixed. Lower film density on later tests indicates reduced developer activity.

The fixer solution is also susceptible to deterioration. The use of exhausted developer solution will produce swelling of the emulsion, insufficient hardening and staining

of the radiograph. Activity of the fixer may be checked by measuring the time required for the film to clear. As the film clears, it will lose its diffuse yellow milkiness. Fixing time can be increased to compensate for reduced activity. A full discussion of the subject with pertinent tables appears in **Radiography in Modern Industry.**

ANALYSIS OF RADIOGRAPHS

The amount of detail that can be differentiated on a radiograph is dependent on radiographic contrast and definition. Subject contrast is dependent on difference in thickness, density and material of the subject. These differences are recorded as differences in darkening or density of the radiograph. Within limits greater contrast provides greater detail. For bark samples and most biological subjects contrast is reduced at higher kilovoltages. Scattered radiation reduces a subject contrast. Film contrast is dependent on film type and the density of the radiograph. In films used for radiographing bark samples contrast increases continuously with density. The greatest range of radiation intensities can be recorded at the upper end of the density scale. In this case, the darkest radiographs that can be easily examined should be used. Both Kodak Type AA and M, the films most commonly used for bark samples,are considered high contrast fine grain x-ray films. The processing activity of the developer also affects film contrast.

The sharpness of the outline of an image is described as definition. Two sets of factors; geometric factors and graininess factors, determine definition. Two of the most important geometric factors are the abruptness of changes in subject thickness and close contact between film and specimen. Faster films are inherently more grainy than slower films. Increasing development time to increase film speed will also increase the graininess of the film.

Illuminators For Radiographic Interpretation. Two types of light sources are used for viewing radiographs. Fluorescent light sources provide a viewing area convenient for examining the entire radiograph. Their low unvariable light level makes them unsuitable for detailed study of radiographs. Variable high intensity illuminators are more suitable for detailed radiograph examination. Variable intensity adjustment provides sufficient intensity for viewing dense portions of the radiograph; lower illumination levels reduce loss of detail in thinner sections of the radiograph. High intensity viewers use a photoflood lamp as a light source. The eye is best able to separate brightness differences when the surroundings are at the same light level as the viewing area. Subdued room lighting is preferable to a darkened room. For maximum detail differentation, the area of interest should be masked to reduce glare and extraneous light from the surrounding portion of the radiograph. When using high intensity illuminators do not look at the brightly lighted viewing screen without a radiograph in place. A foot switch on the viewer will prevent this problem.

Interpreting Radiographs. In most bark beetle brood sample studies a fixed size sample is counted on each bark section. This is most conveniently accomplished on irregular samples by placing an overlay of the required size over the radiograph. Temporary grids and overlays can be made from scale drawings using transparency sheets available for many copying machines. For critical or permanent users, grids may be drawn at an enlarged scale and photographically reduced and printed on acetate by a printer.

A grid with squares eight-tenths of an inch on a side has been found the most useful in determining the area and counting bark beetle brood. Each square on the grid has an area of .004 of a square foot. A portion of the grid is subdivided into .001 square foot blocks. This grid is extremely useful in counting brood as well as measuring the sample area. The .004 square foot blocks are small enough to provide an accurate area estimate yet large enough to provide a good counting unit for dense brood samples. Using the grid and a tally counter, the interpreter can rapidly and systematically scan the radiograph (Fig 24).

Interpreters must receive adequate training to accurately interpret radiographs. In training, the interpreter should be introduced to the principles of radiography. He must understand the effect of radiopacity on tone and the distortion introduced by the position of the specimen and x-ray beam when the exposure was made. Training is most effective when the interpreter can dissect the fresh sample represented by the radiograph. The interpreter's progress can be checked by having him interpret radiographs for which detailed map overlays have been previously made. A file of radiographs with overlays should be built up representing a variety of brood densities and bark thicknesses.

Figure 24. Interpreter estimating brood density on a radiograph on high intensity illuminator with an overlay grid.

Shape, form and location are the keys to identifying x-ray inclusions. Much of the information in this section is from Berryman's 1964 paper, "The Identification of Inclusions in Western Pine Beetle Infested Bark." Magnification is often helpful in identifying inclusion. A reading glass or 10 power magnifier will aid interpretation. For detailed study, a stereo microscope may be mounted for use with the radiograph illuminator. Eggs and early instar larvae are difficult to locate and accurately count. Later stages are usually readily identifiable when exposed laterally in side view. Larvae developing in thick bark may travel laterally; in this position it is hard to separate the life stages. Developing southern pine beetles are more radio opaque than most material in bark radiographs. They register as light images on a dark background. Insect galleries and bark fissures have less thickness than the surrounding bark and register darker. Holes made by egg laying adults that penetrate the entire bark thickness will register as a dark tone. Pitch is fairly radio opaque and will register as a light tone. Pitch in bark beetle galleries may be mistaken for brood especially in radial views. The typical scolytid form of developing pine beetle larvae is easily recognized in radiographs. The head capsule, enlarged thoracic area and curved body are easily recognizable. The head capsule is the most characteristic feature in dorsal view. Viewed head-on, the larvae appear as a light circle on a dark background. The pointed wing pads and the pointed posterior clearly identify the pupae in lateral view. In dorsal view, the pupae's general shape and pointed posterior are identifying characteristics. The characteristic shape of the head and the presence of the thoracic-abdominal junction identify adults in lateral and dorsal views. The legs may often be seen in lateral views. Adults are not readily separable from pupae in head-on views. Both appear as light circles with a notch chipped out against a dark background. The extent of egg galleries, pupal chambers and emergence holes are additional indicators of the degree and success of beetle attack.

The larvae of the major southern pine beetle predators, clerids and ostomids, are easily recognized in radiographs. Their own tunnels are narrow and do not show up on most radiographs.

With experience, secondary insects, ips beetles, and parasites can be identified. When an unfamiliar inclusion is found, it should be recovered by dissecting the bark sample. A notation should be made on the radiograph; its envelope and radiograph logged. These radiographs, along with the preserved specimens, will provide material for training interpreters.

DATA ANALYSIS

The aim of brood density sampling from southern pine beetle infested trees is to determine the average brood density per square foot for the area being sampled. Average brood density can be a key factor in predicting population fluctuations in southern pine beetle population. Data on brood density have proven a reliable indicator of population fluctuations for the mountain pine beetle (Knight, 1960). Brood density figures should not be taken as direct measure of the number of beetles present; but rather as an index of insect activity.

Much work remains to be done in the data analysis of radiographic sampling of southern pine beetle brood. At present, only the arithmetic means and the 90 percent confidence interval are computed. Even these simple computations can become cumbersome on a desk calculator. This is especially true when averages from several samples on a tree as well as per spot and county are required in a district or state survey. Additional problems arise when data from several surveys must be compared. These computations can be efficiently handled on a digital computer. A computer program

(SERDEK) for analyzing brood and predators of the western pine beetle has been developed by Berryman (1965) for population studies. The program was written in FORTRAN IV for use on an IBM 7040/7094 direct coupled computer. This program or an adaptation, could be used in studies of the southern pine beetle.

SIGNIFICANCE OF SURVEY DATA

Sound pest control programs are dependent upon knowing what a particular pest is likely to do next. The foregoing sections covered some of the methods used to gather information on the southern pine beetle. The following is a brief discussion of how this information can be used to make a biological evaluation.

In essence, a biological evaluation is the synthesis of aerial and ground survey data, environmental data, and perhaps most important, the entomologist's knowledge of biology and ecology of the beetle and its host into a tenable theory on the trend of the insect population. Because of the many variables involved, some known, but many unknown, it is impossible to formulate hard and fast rules which can be applied to predict the course of a southern pine beetle population. For this reason today's biological evaluations are more art than science and, as such place considerable demands on the entomologist's skill in selecting the factor or group of factors which are primarily responsible for directing the course of the beetle population at any one particular time and place.

To guide him in this selection the entomologist must look carefully at all of the available data. Perhaps most significant are data which indicate possible changes in the physiological condition of the host. It is widely held that environmental conditions which adversely affect the host are necessary in the development of southern pine beetle epidemics. From aerial and ground survey data the geographic range and severity of attack can be readily determined. Weather records from points both within and outside the outbreak area can then be checked for deviations from the normal precipitation and temperature patterns. This data, however, must be looked at with knowledge of the host's requirements, and soil types involved to be really meaningful.

The most significant statistic obtained from aerial and ground surveys is the number of infested trees per M acres of host type. This figure provides the best estimate of the relative size of the insect population and its capacity for future destruction. Interpretation of specific levels of infestation, however, must be based on knowledge of how the insect and its host react to changing environmental conditions. Thus, what might be considered a dangerous population level in one area at a specific time of year might be considered of little or no importance in another.

The long term trend of an epidemic cannot be determined by one survey. To establish trend it is necessary to have comparable data from several surveys; however, data from one survey sometimes is sufficient to predict short term trends through the ratio of green and fading to red-topped trees. Normally a rapidly expanding bark beetle population is characterized by a high proportion of green and fading infested trees in comparison to abandoned red-topped trees. This is a useful indicator during the summer months when most tree species begin to show foliage symptoms while the beetle broods are still developing. Depending on the geographic location and tree species it could be misleading during cold months when foliage color tends to be either red or green. Surveys made during these months should therefore include extensive ground checks for green infested trees. Even so they are likely to underestimate both the size and vigor of the beetle population. Accurate estimates of bark beetle brood density provides useful information on the biology of the insect. These density measurements are usually stated as number of insects per square foot of bark and are useful in making trend predictions. Changes in brood density reflect changes in the parasite-predator complex, in inter and intraspecific competition and possible changes in the suitability of the host material. These factors must be considered along with other environmental factors to provide sound information on how fast the populations are increasing or decreasing.

In summary, no single factor or group of factors can consistently be held responsible for the sudden outbreaks of declines characteristic of southern pine beetle populations. Only through a thorough understanding of the insect-host-environment relationship will reasonably accurate predictions be possible.

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